N° d'enregistrement
au C.N.R.S. A.O. II292

Article principal
recouvrant en partie
la THESE présentée
pour l'obtention

du grade de DOCTEUR ES SCIENCES
Mention : Sciences Naturelles

A L'UNIVERSITE PARIS VI

par Monsieur André TOULMOND

Sujet de la Thèse :

" Recherches sur la Physiologie Respiratoire de l'Annelide Polychete Arenicola Marina (L.)".

Soutenue le : I2 juin I975

Devant la commission d'Examen composée de :

MM. M.FONTAINE
P.DRACH
J.BERGERARD
P.DEJOURS
G.ECHALIER

RESPIRATION PHYSIOLOGY

SCOPE OF THE JOURNAL

RESPIRATION PHYSIOLOGY publishes original articles or notes concerning respiration taken in a broad sense: physiology of the body considered as a system of gas exchange. Accordingly the journal covers: gas exchange in lungs, gills, skin and tissues; diffusion; gas transport by blood; mechanics of breathing; respiration at rest and in exercise; respiration at sea level, at low pressure (altitude), at high pressure (diving); respiration at different ambient temperatures; foetal and adult respiration; control of respiration; comparative respiratory physiology.
Articles on original methods or techniques in respiration physiology and theoretical papers as well as those dealing with new research findings are also considered.

NOTE TO AUTHORS

The following technical rules should be followed:

Preparation of Text
(a) *Manuscripts* should be typewritten, double spaced, with wide margins. The original and one carbon copy including two sets of figures and tables should be sent to the Editor:

Dr. Pierre Dejours, Laboratoire de Physiologie Respiratoire, CNRS, 23 rue Becquerel, 67-Strasbourg 3, France.

(b) *Title page*. Full title (not to exceed 85 characters including spaces between words), list of authors, laboratory of origin, running title (not exceeding 50 characters including spaces), mailing address.
(c) *Abstract*. An abstract not exceeding 170 words and stating what was done, what was found, and what was concluded, should be typed on a separate sheet. It will appear at the beginning of the article.
(d) Standard nomenclature should be used throughout; unfamiliar or new terms, arbitrary abbreviations, and trade names should be defined when first used. Unnecessary abbreviations and symbols are to be avoided. The most frequently used symbols and abbreviations are listed below.
(e) Legends for illustrations and footnotes should be typed on separate sheets, double spaced. Footnotes, to be consecutively numbered in superscript through the text, should be used as little as possible.

References in the text to literature cited should be given by the name of the author(s), followed by the year of publication in brackets. The reference list, alphabetically arranged and typed double spaced on separate sheets, should use the following forms:
Euler, U. S. von, G. Liljestrand and Y. Zotterman (1939). The excitation mechanism of the chemoreceptors of the carotid body. *Skand. Arch. Physiol.* 83: 132–152.

continued on inside back cover

Respiration Physiology is published once each six weeks.

The subscription price of a volume of about 400 pages is Gld. 74.—, per volume, post-free.

Subscriptions should be sent to the publishers, **North-Holland Publishing Company, P.O. Box 211, Amsterdam** or to any subscription-agent.

Claims for missing numbers should be made within two months, following the date of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

© North-Holland Publishing Company 1973. All Rights Reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the Copyright owner.

Printed in The Netherlands

UNIV. LEIDEN BIBL.

UNIV.
LEIDEN
BIBL.

Respiration Physiology (1973) **19**, 130–144; *North-Holland Publishing Company, Amsterdam*

TIDE-RELATED CHANGES OF BLOOD RESPIRATORY VARIABLES IN THE LUGWORM *Arenicola marina* (L.)

ANDRÉ TOULMOND[1]

Laboratoire de Zoologie, 7 quai Saint-Bernard, 75230 Paris Cedex 05; Station Biologique, 29211 Roscoff; Laboratoire de Physiologie Respiratoire, C.N.R.S., 23 rue Becquerel, 67087 Strasbourg C, France

Abstract. During the period of emersion at low tide the intertidal polychaete annelid *Arenicola marina* (L.) decreases its respiratory exchanges with the surrounding medium. In the prebranchial blood at 15 °C, the percentage saturation of the respiratory pigment is low, 13 to 7%, P_{O_2} is about 1 torr and P_{CO_2} varies between 0.8 and 2.4 torr. Essentially anaerobic metabolism then ensures survival; during 4 hr emersion, blood pH gradually decreases from 7.48 to 7.35, and non volatile acid metabolites accumulate.

During the immersion period at high tide, the animal recuperates, and, by normal respiratory exchanges, metabolites of the anaerobic period are eliminated. pH then rises to the value at which the buffer power of the blood hemoglobin is maximum.

The physiological roles played by the hemoglobin are multiple. At low tide, hemoglobin constitutes only a very limited oxygen reserve, and its importance as the carrier of oxygen in combined form is slight, perhaps nul. By contrast, it is then a major factor in the acid base regulation of the blood. At high tide, the main function of hemoglobin is the transport of oxygen from the surrounding medium toward the cells.

Acid-base balance
Anaerobic energy metabolism
Aquatic respiration
Buffer value
Hemoglobin
Tidal cycle

The marine lugworm *Arenicola marina* (L.) inhabits almost exclusively the intertidal zone, where it often constitutes very large populations. This species constructs rather complex burrows in the sand (Wells, 1945), through which the animal forces a current of water serving respiratory (Wells, 1949) and nutritional needs (Krüger, 1957). At low tide, the burrow cannot be normally irrigated, and the lugworm is then subject to conditions quite different from those existing at high tide, sometimes for more than 9 hr of the $12\frac{1}{2}$-hr tidal cycle[2]. In the closed circulatory system of *Arenicola*, the blood carries a hemoglobin of high molecular weight (*ca.* 3,000,000) in dissolved form.

Accepted for publication 30 May 1973.

[1] Reprint request: A. Toulmond, Laboratoire de Zoologie, 7 quai Saint-Bernard, 75230 Paris Cedex 05, France.

[2] The mean tidal cycle of 12 h 20 is here taken as 12 h 30 for the purpose of simplification.

The present work describes the tide-related changes of certain variables associated with the respiratory function. Measurements were made on prebranchial blood collected at various times during the tidal cycle.

Methods

Lugworms were collected at the exit of the Vieux Port of Roscoff (Nord-Finistère, France), and on the nearby shore at Penpoull, in August, September and October 1972. During this period, the water temperature varied between 15 and 17 °C.

The animal dug out of the sand was placed on gauze. A dorsal incision along the abdominal region exposed the gut. Coelomic liquid was mopped up with the gauze. Blood in the ventral vessel, from which all the efferent branchial vessels branch off, was sucked into a glass micropipette made from a 20 cm long Radiometer capillary. The sealed pipette was placed on an aluminum container set in melting ice. Mean duration of this procedure was 74 sec.

Measurements were made within 4 hr of blood collection. Preliminary experi-

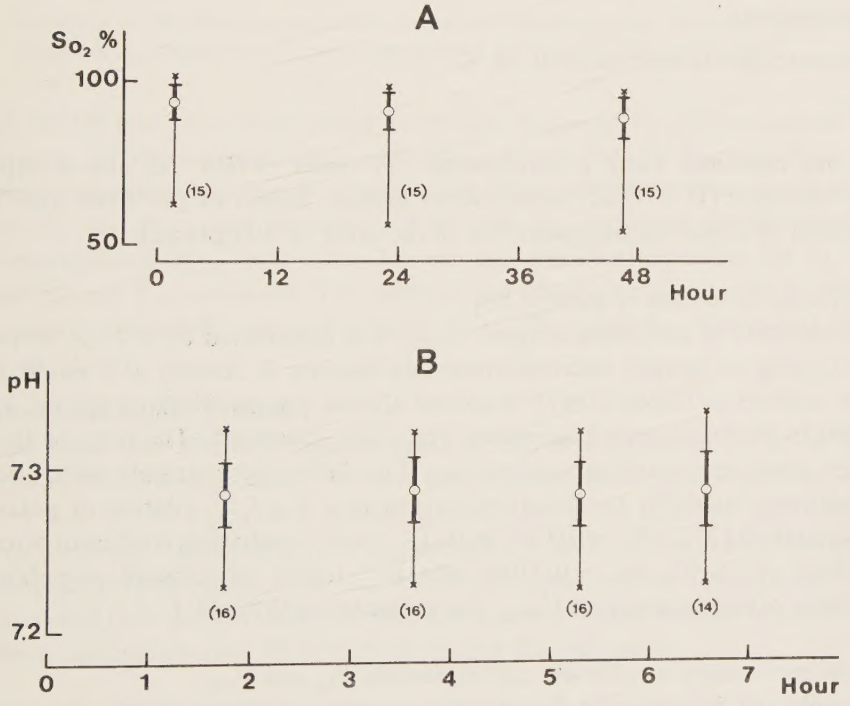

Fig. 1. Variations of percentage saturation of pigment, S_{O_2} (A), and of pH (B), as a function of time, measured on individual blood samples kept on melting ice. Blood samples were collected during the first half-hour of the time scale from 15 (S_{O_2}) and 16 (pH) animals maintained in running sea water. Temperature of measurements = 15 °C; (N) = number of measurements; vertical bar = S.E. × t (Student) for (N) and for P = 0.05; vertical bar between crosses = range of values.

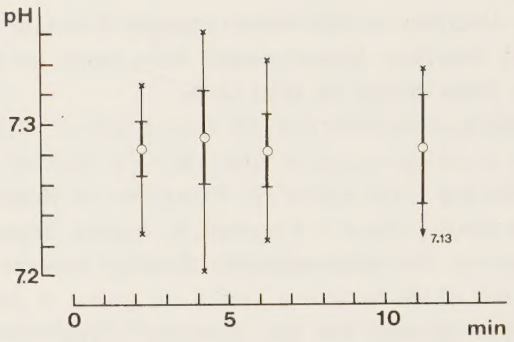

Fig. 2. Variations of blood pH as a function of time elapsed between removal of animals from water and end of blood collection. Four series of 16 animals kept in running sea-water. Temperature of measurements = 15 °C. For other symbols, see fig. 1.

ments confirmed that during this interval, pH and pigment saturation did not appreciably change (fig. 1), and that the pH of the blood in the sampling capillary corresponded effectively to the pH of the circulating blood (fig. 2).

MEASUREMENTS

All measurements were made at 15 °C.

pH

pH was measured using a Radiometer pH meter (PHM 72) and a capillary microelectrode (G 297/G2) standardized against buffers of pH 6.900 and 7.445. Precision of single measurements was of the order of 0.01 pH unit.

Percentage saturation of pigment, S_{O_2}

Concentration of combined oxygen, C_{HbO_2}, was determined on a 20 μl aliquot of blood, using an oxygen microelectrode (Radiometer E 5046/0) in a modification of the method of Tucker (1967). A second aliquot was equilibrated against air for 15 min in an Astrup microtonometer, and $C^{max}_{HbO_2}$ measured. The ratio of the two values gives percentage saturation, S_{O_2}. The following constants were used in calculations: solubility coefficient of oxygen in a 6 $g \cdot L^{-1}$ solution of potassium ferricyanide at 15 °C, $\alpha F_{O_2} = 0.00197\ mM \cdot L^{-1} \cdot torr^{-1}$; solubility coefficient of oxygen in blood at 15 °C, $\alpha b_{O_2} = 0.00164\ mM \cdot L^{-1} \cdot torr^{-1}$ (Toulmond, unpublished). Precision of measurement of C_{HbO_2} was evaluated at 0.1 $mM \cdot L^{-1}$.

Partial pressures of oxygen and carbon dioxide, P_{O_2} *and* P_{CO_2}

P_{O_2} could not be measured directly with precision, owing to the small quantities of blood available, and the low physiological values of P_{O_2}. Determination of P_{CO_2} by Astrup's (1956) method would have been particularly difficult: the buffering power of *Arenicola* blood between pH 7.3 and 7.6 varies widely and abruptly. The properties of the respiratory pigment responsible for these variations (Toul-

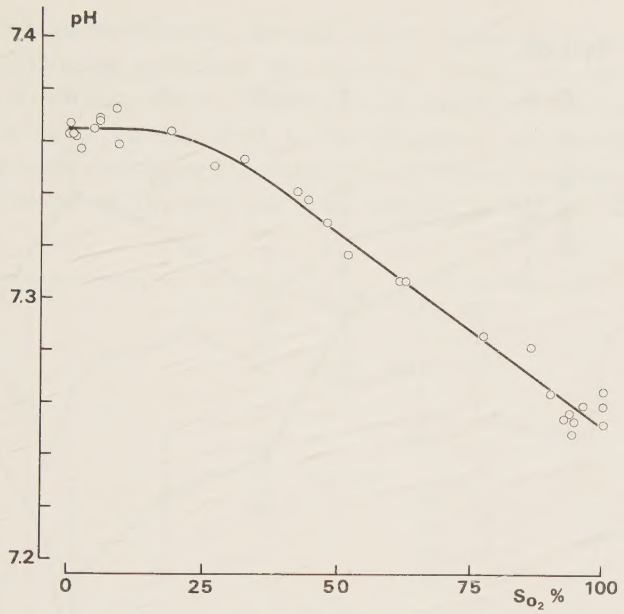

Fig. 3. Variations of pH of one pooled blood sample as a function of the percentage saturation of pigment, S_{O_2}. Temperature of measurements = 15 °C; $P_{CO_2} = 0$; $C^{max}_{HbO_2} = 5.1$ mM·L^{-1}.

mond, 1971b) give a particular shape to the log $P_{CO_2} = f(pH)$ relation at low P_{CO_2} (Toulmond, 1970b) and non-linear $pH = f(S_{O_2})$ curves (fig. 3). P_{O_2} and P_{CO_2} were therefore determined together by equilibrating blood samples against gas phases of known P_{O_2} and P_{CO_2} until the values of pH and S_{O_2} measured directly as above were encountered. P_{CO_2} then being known, the equilibration curve for O_2 was completed, and P_{50} evaluated. The different gas phases were prepared by mixing pure gases in Wösthoff pumps. Precision of P_{O_2}, P_{CO_2} and P_{50} values was of the order of 0.2 torr.

Protein concentration, [Protein]

2 ml of Levin and Brauer's biuret reagent (1953) was added to 20 μl of blood mixed with 3 ml of distilled water. At room temperature the reaction is complete in 2 to 3 hr. A fine brown precipitate, probably denatured heme residues, was removed by centrifugation. Optical density of the supernatant liquid placed in a quartz cuvette was measured at 540 mμ in a Jobin and Yvon spectrophotometer. The standard curve was prepared from pooled *Arenicola* blood whose dry weight protein concentration had been determined after 72 hr dialysis against distilled water.

EXPERIMENTAL PROTOCOLS

Three types of experiments were performed.

Experiment 1

Seven stations, A to G, were spotted from the top to the bottom of the

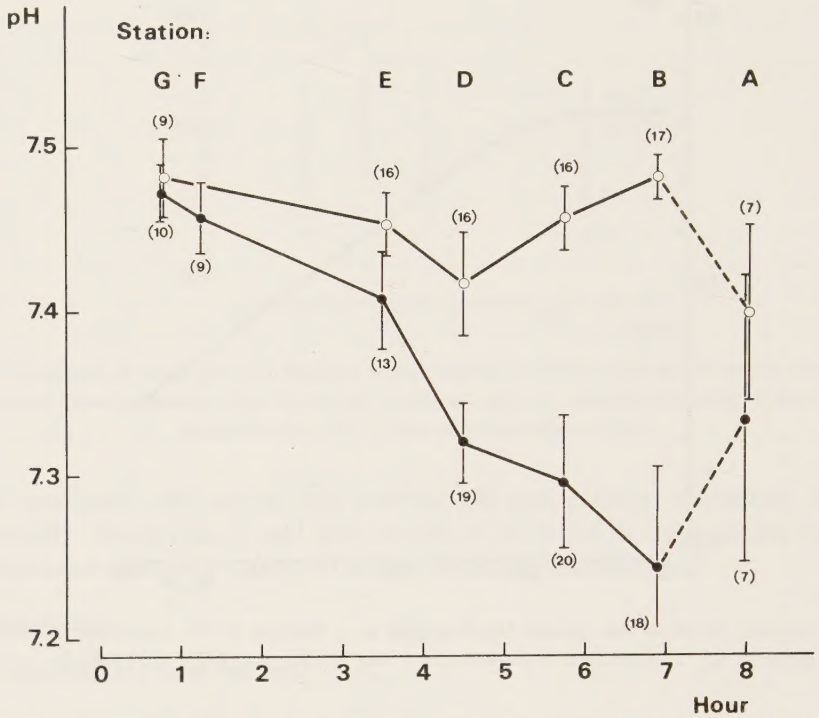

Fig. 4. Variations of blood pH as a function of duration of emersion. Blood samples collected on Penpoull Stations A-G. –○–, blood sampled within 45 min *after emersion;* –●–, blood sampled within 45 min *preceding immersion* of the station. All measurements at 15 °C. For other symbols, see fig. 1.

Station A situated high on the shore differs from the others by its particular edaphic characteristics. Its sand is fully oxidized down to 30 cm and is constantly perfused with aerated brackish water. The very dense population of *Arenicola* is composed of small, recently hatched animals (Newell, 1948).

intertidal zone at Penpoull. On the days measurements were made, these stations were out of water respectively 8 hr, 6 hr 55, 5 hr 45, 4 hr 40, 3 hr 30, 1 hr 15 and 0 hr 45. At each station, 7 to 20 blood samples from as many animals were collected within 45 min *after emersion*, and within 45 min *preceding immersion*. pH was measured on each sample; the results are summarized in fig. 4.

Experiment 2

A station chosen at the exit of the Vieux Port of Roscoff emerged for about 4 hr 30 of the tidal cycle. The first 4 hr of emersion was divided into 1-hour periods, H1 to H4. During each hourly period, blood of 16 specimens was collected at the shore. In the laboratory, the 16 samples were pooled and mixed anaerobically in a glass syringe. pH and S_{O_2} were immediately measured on the mixed blood, and P_{O_2}, P_{CO_2}, P_{50} and [Protein] determined later.

Experiment 3

This experiment was designed to follow the course at high tide of the variables

studied at low tide in experiment 2. Because it was impossible to sample blood at the shore, the following procedure was adopted: during the last half-hour of emersion of the Vieux Port station, 25 animals were quickly collected and placed in a container of sea water, brought back to the laboratory and transferred into an aquarium of sea water circulating at constant flow. Defecated sand was removed as it appeared. The blood of 16 specimens was sampled during the first hour, H5,

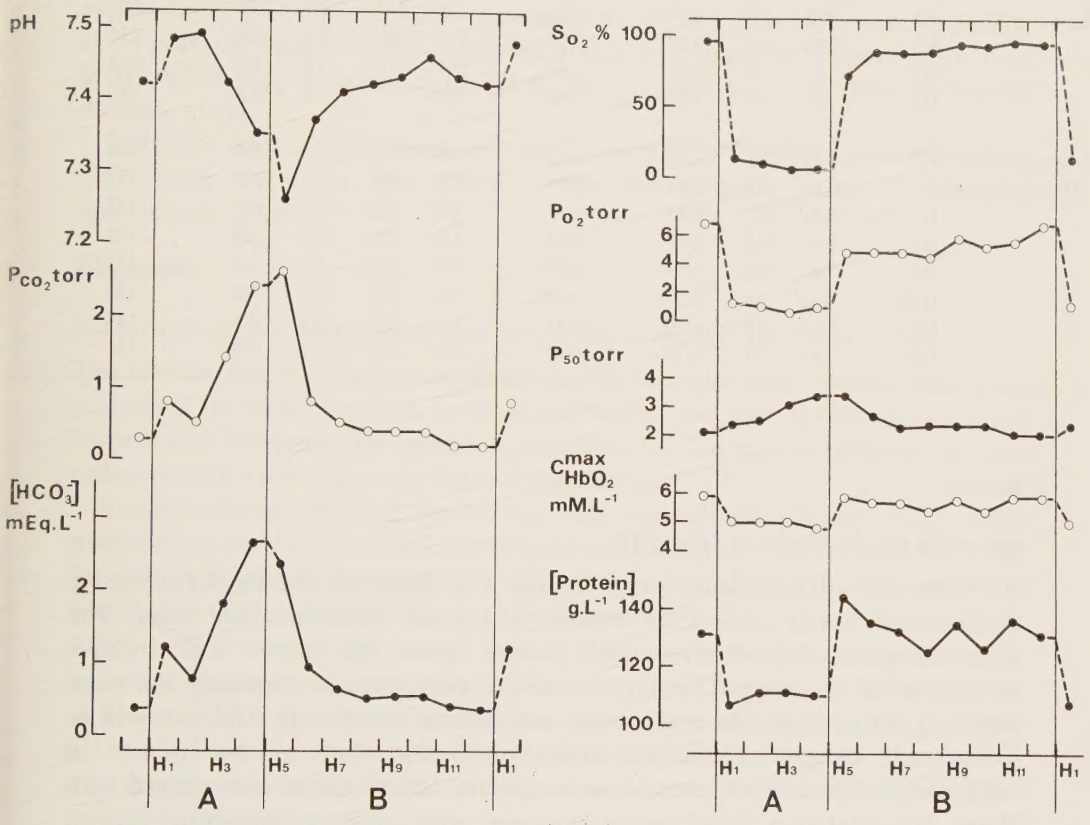

Fig. 5. Variations of blood pH, P_{CO_2} and [HCO_3^-] during a 12-hr tidal cycle. All measurements at 15 °C. A, emersion; B, immersion.

Fig. 6. Variations of S_{O_2}, P_{O_2}, P_{50}, $C^{max}_{HbO_2}$ and [Protein] during a 12-hr tidal cycle. See legend of fig. 5.

corresponding to the 5th hr after low tide at the collecting station. On the following days, the procedure was repeated, except that blood was taken 1 hr later each day, up to the 8th hr since placement of the animals in the aquarium (samples H6–H12). Samples H5 to H12 were treated and analyzed as in Experiment 2. The results of Experiments 2 and 3 are given in figs. 5 and 6 and in table 1.

TABLE 1

Respiratory variables in blood of *Arenicola* during the tidal cycle. Measurements made on pooled blood from 16 animals. Temperature of measurements = 15 °C; $\alpha b_{CO_2} = 0.047$ mM · L^{-1} · torr^{-1}; $pK'_1 = 5.98$ (Toulmond, unpublished).

	Hour No	pH	P_{CO_2} torr	$[HCO_3^-]$ meq · L^{-1} (calc.)	C_{CO_2} mM · L^{-1} (calc.)	P_{O_2} torr	S_{O_2} %	P_{50} torr	$C^{max}_{HbO_2}$ mM · L^{-1}	[Protein] g · L^{-1}
Low tide	H1	7.48	0.8	1.20	1.24	1.2	13	2.4	5.0	107
	H2	7.49	0.5	0.78	0.80	1.0	10	2.5	5.0	111
	H3	7.42	1.4	1.82	1.88	0.6	6	3.1	5.0	111
	H4	7.35	2.4	2.65	2.76	0.9	7	3.4	4.8	110
Experimental "High tide"	H5	7.26	2.6	2.33	2.45	4.8	72	3.4	5.9	144
	H6	7.37	0.8	0.93	0.97	4.8	89	2.7	5.7	135
	H7	7.41	0.5	0.65	0.67	4.8	88	2.3	5.7	132
	H8	7.42	0.4	0.52	0.54	4.5	89	2.4	5.4	125
	H9	7.43	0.4	0.54	0.56	5.8	94	2.4	5.8	135
	H10	7.46	0.4	0.57	0.59	5.2	93	2.4	5.4	126
	H11	7.43	0.3	0.39	0.41	5.5	97	2.1	5.9	137
	H12	7.42	0.3	0.39	0.40	6.8	95	2.1	5.9	131

Results

BEHAVIOR OF *Arenicola* AT LOW TIDE

At all the collecting stations (except Station A at Penpoull; see legend of fig. 4), the lugworm lies in its burrow containing hypoxic interstitial sea water. The immobile animal was closely enough pressed against the burrow wall to leave an imprint of its rings. During this period, the worm is digesting the sand absorbed during high tide, and moves only seldom to defecate. Thus its activity is extremely reduced. The blood circulation is also slow: at the moment of collection, the dorsal and ventral vessels and the lateral hearts were gorged with blood. This dilatation of the vessels was never observed in the specimens kept in running water.

TIME COURSE OF RESPIRATORY VARIABLES DURING LOW TIDE

From the results of Experiment 1 at Penpoull (fig. 4), it can be clearly seen that the pH of prebranchial blood decreased during low tide, this drop being the greater the longer the period of emersion of the animal. Experiment 2 (figs. 5 and 6) shows that during 4 hr of emersion, blood pH went from 7.48 to 7.35. Concomitantly, P_{CO_2} increased from 0.8 to 2.4 torr. During the entire low-tide period, P_{O_2} remained at about 1 torr, and S_{O_2} was low, decreasing from 13 to 7%. Because of the developing acidosis, P_{50} moved from 2.4 to 3.4 torr, thus confirming the existence of a functional Bohr effect (Toulmond, 1970a).

TIME COURSE OF RESPIRATORY VARIABLES DURING HIGH WATER

In Experiment 3, immersion of the animals, corresponding to wetting by the rising tide, brought an abrupt increase in blood P_{O_2} which stabilized at 5 to 6 torr. This value for P_{O_2}, low compared to that of the water bathing the gills (P_{O_2} *ca* 140 to 150 torr), is confirmed by direct measurements made on other blood samples (P_{O_2} = 8.8 torr; t × S.E. = 1.7; p = 0.05; N = 10; pH = 7.26). S_{O_2} rapidly rose, reaching 70% at H5 and leveling at about 90%. This increase in S_{O_2} entailed a new decrease of pH (see fig. 3), a drop probably responsible for the temporary maintenance of P_{CO_2} at its previous high level. pH then rose evenly to stabilize at a lower value than in H1, while P_{CO_2} decreased from 2.4 to 0.5 torr, and P_{50} went down to resume its value at H1.

The mean value of $C^{max}_{HbO_2}$ during emersion, 4.95 mM · L $^{-1}$, differs considerably from that during immersion, 5.70 mM · L^{-1}. The corresponding values for [Protein] are 110 and 133 g · L^{-1}.

Discussion

VARIATIONS OF THE BLOOD ACID-BASE EQUILIBRIUM DURING THE TIDAL CYCLE

The pattern of pH variations observed during the tidal cycle reveals that 1) an acidosis of the blood develops in the animal during low tide; 2) this acidosis is the more intense the longer the period of emersion; 3) it disappears progressively and rather rapidly with immersion of the animal by tide.

As the acidosis develops, clearly P_{CO_2} also rises. If the pH and P_{CO_2} values measured on the Vieux Port animals (pooled samples; table 1) are substituted in the Henderson-Hasselbalch equation, it can be seen that during the 4-hr emersion period, the total concentration of CO_2 in the blood, C_{CO_2}, passes from 1.24 to 2.76 mM · L^{-1} mainly by a rise of $[HCO_3^-]$. However, this CO_2 accumulation is not alone responsible for the observed decrease of pH, since, when blood samples at H4 and H12 are equilibrated against gas phases of increasing P_{CO_2}, blood pH at H4 is always less than that at H12, whatever the P_{CO_2} (fig. 7). Thus, at low tide, the variation of bicarbonate concentration is accompanied by changes in non-volatile blood components.

Accumulation of CO_2 in the blood at low tide must result from a decreased exchange of respiratory gases with the surrounding medium. The corresponding values of S_{O_2}, so low compared to those at high tide, reinforce this idea. Such a reduction of respiratory exchanges suggests that metabolism is at least partially anaerobic at low tide, a possibility supported by Hecht's finding (1932) that *Arenicola* can withstand 9 days of complete anaerobiosis. Accumulation of acid metabolites could then explain the drop in pH observed at low tide. The nature of such metabolites is unknown. Dales (1958) has found that experimentally induced anaerobiosis of *Arenicola marina* causes a reduction of glycogen concentration in the body wall muscles without a local increase of lactic acid concentration. Coles (1970) in his studies on the oligochaete *Alma emeni* has shown that the inter-

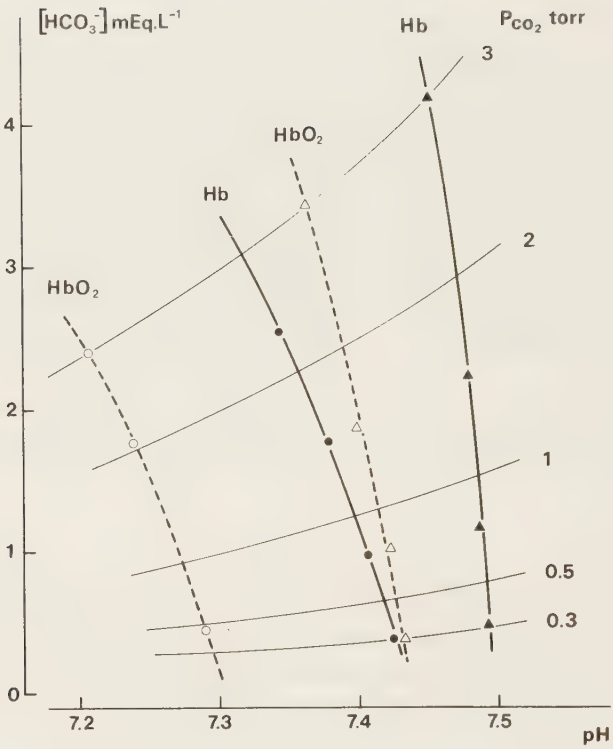

Fig. 7. $HCO_3^- = f(pH)$ diagram of *in vitro* equilibration at 15 °C of blood from H4 (end of emersion ● ○) and from H12 (end of immersion ▲ △) against various P_{CO_2}, with (○ △) or without (● ▲) oxygen. It may be seen that the H4 curves are shifted down the pH scale compared to the H12 curves. $pk_1' = 5.98$ (Toulmond, unpublished); $\alpha b_{CO_2} = 0.047$ mM · L^{-1} · torr^{-1} (Toulmond, 1970b).

mediary products of anaerobic metabolism are extremely numerous and varied in annelids. Identification of such metabolites is at present being attempted for *Arenicola marina*.

Whatever these acid metabolites may be, during high tide they are eliminated progressively, as can be seen from the pattern of pH variation in fig. 5, and rather rapidly, since pH stabilizes 3 hr after immersion of the animal. Perhaps they are directly "excreted" through the body wall, as suggested by Dales (1958), or they might be used in the resynthesis of, for instance, glycogen. This possibility is less likely, since Borden (1931) was unable to demonstrate payment of an oxygen dept in the lugworm exposed to 2 hr of experimental anoxia.

Carbon dioxide accumulated during low water is eliminated as normal respiratory exchanges are resumed at high tide. Blood bicarbonate concentration decreases and, after a few hours, blood pH levels off at 7.42, a value at which the buffering power (15 °C) of the oxyhemoglobin is maximum (Toulmond, 1971b). pH at H1 is a little higher, 7.48, and so is the mean value observed at Penpoull, Stations B–G, at the beginning of low tide ($pH = 7.46$, $N = 74$, $t \times S.E. = 0.01$, $p = 0.05$). The

difference is due to the changed oxygenation of the respiratory pigment: $S_{O_2} = 13\%$ at H1, and 96% at H12. The animal's respiratory exchanges appear to decrease from the very beginning of emersion. The resulting abrupt drop of S_{O_2} provokes a transitory blood alkalosis, in spite of a slight rise of P_{CO_2} (figs. 5 and 6). Thus the blood at the beginning of the emersion period is in a state to best resist acidosis. The buffering power of deoxyhemoglobin which is maximum at pH 7.48 for a temperature of 15 °C (Toulmond, 1971b), is about 4 times that of oxyhemoglobin. The respiratory pigment thus plays the role of a buffer storage at low tide, and contributes to limiting the effects of anaerobic metabolism on blood pH.

BLOOD OXYGEN TRANSPORT DURING THE TIDAL CYCLE

In the animals kept in running water (P_{O_2} *ca* 150 torr), P_{O_2} of prebranchial blood was about 6 torr for $S_{O_2} = 88$–97%. If P_{O_2} of postbranchial blood is taken as 140 torr for $S_{O_2} = 100\%$, and if $C^{max}_{HbO_2} = 5.7\ mM \cdot L^{-1}$, then, by calculation, 44 to 76% of the consumed oxygen is transported in combined form, and the rest dissolved. Thus, contrary to what Wald and Allen (1957) have held, a respiratory pigment with a high affinity for oxygen can participate in the transport of this gas: in *Arenicola marina*, $P_{50} = 2$ torr, pH = 7.5, t = 15 °C (Toulmond, 1970a). This estimate also contradicts the common opinion (Eliassen, 1955; Jones, 1972) that such a pigment does play a role in oxygen transport only when ambient P_{O_2} is low. In fact, it is quite possible that when the animal is in its burrow, not artificially placed in running water, the proportion of consumed oxygen transported in combined form may be greater than the above calculation would suggest. In the first place, the water irrigating the worm's burrow at the shore comes from liquid layers near the sediment, and it is not obvious that this water will always be saturated with oxygen (P_{O_2} *ca* 150 torr). In the second place, each of the 13 gill pairs is bathed by water of lower P_{O_2} the farther away from the burrow entrance a given gill pair is. Van Dam (1938) has shown experimentally that *Arenicola* can extract 30 to 50% of the oxygen passing through the burrow. In the same experimental conditions, we have found that for $Pw_{O_2} = 158$ torr at the entrance of an artificial burrow, Pw_{O_2} at the exit can be quite low: 61 ± 9 torr ($N = 44$, $p = 0.01$; range 15–120 torr). In normal conditions of ventilation, mean P_{O_2} of the postbranchial blood must then be much less than 140 torr, and the quantity of oxygen transported in dissolved form very low.

Under certain conditions, the oxygen consumed by the animal could come only from oxyhemoglobin. The ventilatory activity of *Arenicola* is rhythmic. Periods of ventilation alternate with shorter rest periods during which the animal eats or enlarges its burrow (Van Dam, 1938; Wells, 1949; Krüger, 1964). The P_{O_2} of unventilated burrow water must decrease rapidly, and aerobic metabolism would then be entirely dependent on oxygen transported in combined form. The pigment would here play the "emergency role" attributed to it by Jones (1972).

At low tide, the conditions are quite different. The percentage saturation of the prebranchial blood pigment is low and decreases progressively from 13 to 7% for a

P_{O_2} of about 1 torr. These findings corroborate Manwell (1963), who has described the hemoglobin of blood collected at low tide from *Arenicola pacifica* and *A. vagabonda* as being practically desaturated in oxygen. Since the characteristics of postbranchial blood in the emerged animal are unknown, it cannot be affirmed that oxygen transport stops completely during this period, although evidence cited above suggests that it is so low that P_{O_2} of prebranchial blood is little above 1 torr. Aerobic and anaerobic metabolism could nevertheless coexist at low tide. Weber and Pauptit (1972) have shown for *Arenicola marina* that oxygen is relayed between hemoglobin and the muscular cell by two myoglobins of P_{50} between 0.6 and 1.2 torr (pH = 7.38; t = 20 °C). Oxygen could therefore theoretically be extracted from the blood at very low pressure.

Finally, Eliassen (1955) reports that in *Arenicola marina*, the quantity of oxygen in the blood at low tide is always about equal to $C^{max}_{HbO_2}$, whatever the duration of emersion. That these findings are in complete contradiction with ours is probably to be explained by edaphic conditions differing from those observed at all our collecting sites except Station A at Penpoull. At Penpoull A, the worms were immersed almost permanently, and all through the low water period their blood was bright red, indicating it was oxygen-rich. At all the other stations, on the contrary, blood was deep purple from the first hour of emersion, a sign of oxygen desaturation confirmed by low measured values of S_{O_2}.

OXYGEN STORAGE FUNCTION OF THE BLOOD PIGMENT DURING THE TIDAL CYCLE

Noting the high value of $C^{max}_{HbO_2}$ in *Arenicola*, Barcroft and Barcroft (1924) were of the opinion that the pigment saturated with oxygen at high tide becomes a reserve for maintainance of aerobic metabolism during low tide when normal respiratory exchanges are impossible. This hypothesis, based on calculations involving the volume of the circulatory compartment, Vb, oxygen consumption, $\dot{V}_{O_2}$, and $C^{max}_{HbO_2}$, was supported by Borden (1931) and criticized by Wolvekamp and Vreede (1941), Eliassen (1955) and Jones (1972). Using our values (Toulmond, 1971a), we find that in a specimen of 11 ml total volume, of which Vb = 0.7 ml, when $C^{max}_{HbO_2} = 5\ mM \cdot L^{-1}$ and $\dot{V}_{O_2} = 340\ \mu l \cdot hr^{-1}$ at 15 °C (Toulmond, unpublished), the quantity of oxygen combined with pigment cannot provide for more than 14 min of normal aerobic metabolism. Even though at low tide the oxygen consumption of the sluggish animal must be low, Barcroft and Barcroft's hypothesis is too extreme. A value of $S_{O_2} = 13\%$ at the first hour of emersion indicates that the oxygen combined with the saturated pigment is quickly used up, a condition otherwise advantageous in the resistance to blood acidosis (see above).

Eliassen (1955) agrees that the oxygen reserve constituted by the saturated pigment must be exhausted within a few minutes, although the terms used in his calculations are insufficiently precise, notably the high value of Vb (Prosser, 1950; Toulmond, 1971a). For this author, the reserve would come into play only during the rhythmic ventilatory pauses observed at high tide ("rhythmic reservoir function"). This idea complements our conclusions (and those of Jones, 1972) concerning transport of

oxygen by the pigment during the ventilatory pauses of high tide. The very high oxygen affinity of the respiratory pigment ought to mean that O_2 can be extracted at low pressure from the stagnant water of the burrow. This extraction must have its limits, however, and possibly the animal utilizes the little oxygen left in combined form before it again starts to breathe normally.

VARIATIONS OF $C^{max}_{HbO_2}$ AND [PROTEIN] BETWEEN LOW AND HIGH TIDE

The simultaneous increases of $C^{max}_{HbO_2}$ and protein concentration observable at the beginning of the emersion period may be explained in two ways: 1) a liberation of protein from the extravasal tissue, the site of synthesis (Breton-Gorius, 1963) of the respiratory pigment, 2) concentration by loss of water from the circulatory compartment. The first hypothesis seems unlikely, in view of the large amount of protein to be freed rapidly every 12 hr. The second, more reasonable, hypothesis may be understood in terms of Starling's (1909) analysis of the movement of liquid between blood and interstitial compartments, which constitute respectively 6 and 35% of the volume of a 10 ml worm (Toulmond, 1971a). Exchange then takes place between two compartments which are in intimate contact everywhere (Gamble and Ashworth, 1898) and come to an equilibrium in periods of normal activity during immersion. It may be that during emersion the slowing down of the blood circulation is enough to force water from the coelomic into the circulatory compartment, by a simple decrease of the mechanical pressure of blood in the capillaries. The increase in P_{CO_2} is another factor which might intervene: in mammals at least it causes a peripheral vasodilation resulting in a decrease of blood pressure.

Conclusions

The main findings may be summarized fairly simply (fig. 8). Low tide corresponds for *Arenicola* to a period of at least partial anaerobiosis provoked by a decrease of respiratory exchanges with the exterior milieu. The effects of this anaerobiosis on the blood pH are limited 1) by the direct excretion (diffusion) of some anaerobic metabolites (Dales, 1958), 2) by the reduction of activity to a minimum (Van Dam, 1938; Wells, 1949), 3) by the buffering characteristics of the abundant respiratory pigment. High tide, by contrast, is a period of aerobiosis during which the animal feeds actively and eliminates accumulated acid metabolites.

This interpretation, probably valid for many intertidal organisms, is supported by a comparison of the size of *Arenicola* specimens collected at different levels of the intertidal zone. The metabolic efficiency ought to be the greater the shorter the period of emersion, as is the case for the mollusks *Ostrea*, *Mytilus edulis* (Loosanoff, 1942), and *Cardium edule* (Kristensen, 1957). The largest specimens of *Arenicola marina* are collected at the low water of spring tides, where they are so huge that Gamble and Ashworth (1898) took them for a distinct variety. As Wells (1957) has emphasized, it simply means that they live longer and better than worms colonizing the upper levels.

Nevertheless, the density of population in the intertidal zone shows that *Arenicola*

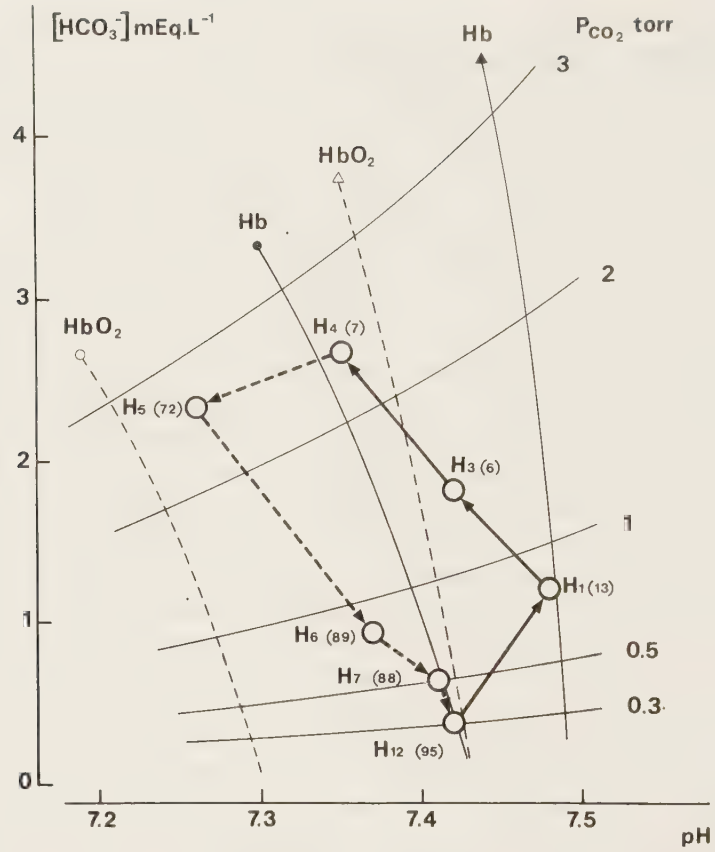

Fig. 8. *In vivo* values of blood pH and $[HCO_3^-]$ for H1, H3, H4, H5, H6, H7 and H12 superimposed on the family of curves of fig. 7. In parentheses, corresponding measured value of S_{O_2}. —, emersion; ---, immersion.

can support long periods of emersion. We feel that this capacity is clearly dependent on the properties of the blood respiratory pigment.

During high tide, the pigment transports oxygen from the exterior milieu to the cells. It contributes to maintenance of a constant blood pH. Towards the end of the normal short ventilatory pauses, it can play the role of an oxygen reserve.

During low tide, the respiratory pigment serves as an oxygen reserve only for a short time, and its role in oxygen transport is probably slight. Practically deoxygenated for all of the critical emersion period, its high concentration and its buffering characteristics suggest that the pigment is a prime factor in the pH regulation of the interior milieu during anaerobiosis. The mollusk's shell is known to play a similar role (*Venus mercenaria:* Collip (1921) and Dugal (1939); *Anodonta cygnea:* Dotterweich and Elssner (1935); *Pila:* Meenakshi (1958)). In vertebrates, the respiratory pigment also intervenes in blood pH regulation, either directly, as all protein, or indirectly, by the release and uptake of protons with oxygenation and deoxygenation ("vertical Bohr effect", Rossi-Bernardi and Roughton (1967) and fig.

3). In *Arenicola marina*, a supplementary buffer effect probably linked, like the vertical Bohr effect, to the rearrangement of the molecular structure of the protein, is restricted to a narrow range of physiological pH values and is the stronger the lower the percentage saturation of the hemoglobin with oxygen.

Acknowledgements

Special thanks are due to the staff of the Station Biologique de Roscoff, Nord Finistère, for the facilities put at our disposal.

References

Astrup, P. (1956). A simple electrometric technique for the determination of carbon dioxide tension in blood and plasma, total content of carbon dioxide in plasma, and bicarbonate content in "separated" plasma at a fixed carbon dioxide tension (40 mm Hg). *Scand. J. Clin. Lab. Invest.* 8: 33–43.

Barcroft, J. and H. Barcroft (1924). The blood pigment of *Arenicola*. *Proc. Roy. Soc. London, B*, 96: 28–42.

Borden, M. A. (1931). A study of the respiration and of the function of hemoglobin in *Planorbis corneus* and *Arenicola marina*. *J. Mar. Biol. Ass. U.K.* 17: 709–738.

Breton-Gorius, J. (1963). Etude au microscope électronique des cellules chloragonènes d'*Arenicola marina* L.: leur rôle dans la synthèse de l'hémoglobine. *Ann. Sc. nat.* 12: 211–271.

Coles, G. C. (1970). Some biochemical adaptations of the swamp worm *Alma emini* to low oxygen levels in tropical swamps. *Comp. Biochem. Physiol.* 34: 481–489.

Collip, J. B. (1921). A further study of the respiratory processes in *Mya arenaria* and other marine molluscs. *J. Biol. Chem.* 49: 297–310.

Dales, R. P. (1958). Survival of anaerobic periods by two intertidal polychaetes, *Arenicola marina* L. and *Owenia fusiformis* Della Chiaje. *J. Mar. Biol. Ass. U.K.* 37: 521–529.

Dotterweich, H. and E. Elssner (1935). Die Mobilisierung des Schalenkalkes für die Reaktionsregulation der Muscheln *(Anodonta cygnea)*. *Biol. Zentr.* 55: 138–163.

Dugal, P. L. (1939). The use of calcareous shell to buffer the product of anaerobic glycolysis in *Venus mercenaria*. *J. Cell. Comp. Physiol.* 13: 235–251.

Eliassen, E. (1955). The oxygen supply during ebb of *Arenicola marina* in the Danish Waddensea. *Univ. Bergen Årbok, Naturvit. Rekke* 12: 1–9.

Gamble, F. W. and J. H. Ashworth (1898). The habits and structure of *Arenicola marina*. *Quart. J. Micr. Sci.* 41: 1–42.

Hecht, F. (1932). Der chemische Einfluss organischer Zerzetzungsstoffe auf das Benthos, dargelegt an Untersuchungen mit marinen Polychaeten, insbesondere *Arenicola marina* L. *Senckenbergiana* 14: 199–220.

Jones, J. D. (1955). Observations on the respiratory physiology and on the haemoglobin of the Polychaete genus *Nephthys*, with special reference to *N. hombergii* (Aud. et M. Edw.). *J. Exp. Biol.* 32: 110–125.

Jones, J. D. (1972). Comparative Physiology of Respiration. London, Edward Arnold Publ. Ltd.

Kristensen, I. (1957). Differences in density and growth in a cockle population in the Dutch Wadden Sea. *Arch. Neerl. Zool.* 12: 351–453.

Krüger, F. (1957). Der Wohnbau von *Arenicola* als Filtereinrichtung. *Naturw.* 44: 597–598.

Krüger, F. (1964). Messungen der Pumptätigkeit von *Arenicola marina* L. im Watt. *Helgol. wiss. Meeresunters.* 11: 70–91.

Levin, R. and R. W. Brauer (1953). The biuret reaction for the determination of protein: an improved reagent and its application. *J. Lab. Clin. Med.* 38: 474–480.

Loosanoff, V. L. (1942). Shell movements of the edible mussel *Mytilus edulis* (L.) in relation to temperature. *Ecology* 23: 231–234.

Manwell, C. (1963). The blood proteins of Cyclostomes. A study in phylogenetic and ontogenetic biochemistry. In: The Biology of Myxine, ed. by Brodel and Fänge. Oslo Univ. Press., pp. 372–455.

Meenakshi, V. R. (1958). Anaerobiosis in the South Indian apple snail *Pila virens* during estivation. *J. Zool. Soc. (India)* 9: 62–71.

Newell, G. E. (1948). A contribution to our knowledge of the life history of *Arenicola marina*. *J. Mar. Biol. Ass. U.K.* 27: 554–580.

Prosser, C. L. (1950). Comparative Animal Physiology. Philadelphia and London, W. B. Saunders Co.

Rossi-Bernardi, L. and F. J. W. Roughton (1967). The specific influence of carbon dioxide and carbamate compounds of the buffer power and Bohr effects in human hemoglobin solutions. *J. Physiol. (London)* 189: 1–29.

Starling, E. H. (1909). The Fluid of the Body. Chicago, Keener.

Toulmond, A. (1970a). La fixation de l'oxygène par le sang chez l'Arénicole [*Arenicola marina* (L.), Annélide Polychète]. *C.R. Acad. Sci. (Paris)* 270: 1368–1371.

Toulmond, A. (1970b). La fixation du dioxyde de carbone par le sang chez l'Arénicole [*Arenicola marina* (L.), Annélide Polychète]. *C.R. Acad. Sci. (Paris)* 270: 1487–1490.

Toulmond, A. (1971a). Détermination du volume des compartiments coelomique et circulatoire chez l'Arénicole, *Arenicola marina* (L.), Annélide Polychète. *C.R. Acad. Sci. (Paris)* 272: 257–260.

Toulmond, A. (1971b). Sur une particularité du pouvoir tampon de l'hémoglobine d'Arénicole [*Arenicola marina* (L.), Annélide Polychète]. *C.R. Acad. Sci. (Paris)* 272: 3184–3187.

Tucker, V. A. (1967). Method for oxygen content and dissociation curves on microliter blood samples. *J. Appl. Physiol.* 23: 410–414.

Van Dam, L. (1938). On the utilisation of oxygen and regulation of breathing in some aquatic animals. *Diss. Phil. Groningen* 7: 1–143.

Wald, G. and D. W. Allen (1957). The equilibrium between cytochrome oxydase and carbon monoxide. *J. Gen. Physiol.* 40: 593–608.

Weber, R. E. and E. Pauptit (1972). Molecular and functional heterogeneity in myoglobin from the Polychaete *Arenicola marina* L. *Arch. Biochem. Biophys.* 148: 322–324.

Wells, G. P. (1945). The mode of life of *Arenicola marina*. *J. Mar. Biol. Ass. U.K.* 26: 170–207.

Wells, G. P. (1949). Respiratory movements of *Arenicola marina* L.: intermittent irrigation of the tube and intermittent aerial respiration. *J. Mar. Biol. Ass. U.K.* 28: 447–464.

Wells, G. P. (1957). Variation in *Arenicola marina* (L.) and the status of *Arenicola glacialis* Murdoch (Polychaeta). *Proc. Zool. Soc. London* 129: 397–419.

Wolvekamp, H. P. and M. C. Vreede (1941). On the gas binding properties of the blood of the lugworm (*Arenicola marina* L.). *Arch. Neerl. Physiol.* 25: 265–276.

continued from inside front cover

Defares, J. G. and I. N. Sneddon (1961). An Introduction to the Mathematics of Medicine and Biology. Amsterdam, North-Holland Publ. Co., pp. 213–215.

Irving, L. (1964). Comparative anatomy and physiology of gas transport mechanisms. In: Handbook of Physiology. Section 3. Respiration. Vol. I, edited by W. O. Fenn and H. Rahn. Washington D.C., American Physiological Society.

To cite several papers by the same author in one year, place small a, b, etc. after year of publication. Please refer to Respiration Physiology as: Respir. Physiol.

Illustrations, Graphs and Tables

(a) All illustrations and graphs, to be referred to as figures, should be numbered in Arabic numerals (fig. 2, 3 ... etc.).
(b) Submit either original drawings in India ink or sharp, contrasty, unmounted photographs on glossy paper. The lettering must be proportional to the size of the illustrations if it is to be legible after reduction (type area 20.5 × 12.6 cm).
(c) Actual magnification of all photomicrographs should be given.
(d) Tables should be numbered separately in Arabic numerals (table 1, 2... etc.).
(e) The approximate position of each figure should be indicated in the margin of the manuscript.

Proofs

Two sets of galley proofs will be sent to the author, to be carefully checked for any necessary changes or printer's errors.

Reprint

Each author receives with his galley proofs a reprint order form which must be completed and returned to the publisher with the proofs. Later orders cannot be filled since reprints are made at the time the journal is printed.

SYMBOLS

The following symbols should be used.

I	Inspired gas	L	Pulmonary
E	Expired gas	b	Blood in general
A	Alveolar gas	a	Arterial
D	Dead space gas	c	Capillary
T	Tidal gas	v	Venous
B	Barometric	$\bar{v}$	Mixed venous blood
F	Fractional concentration in dry gas phase	V	Gas volume
P	Gas pressure in general	$\dot{V}$	Gas volume per unit time
C	Concentration in blood phase	$\dot{Q}$	Blood volume per unit time
S	Saturation	D	Diffusing capacity
R	Respiratory exchange ratio	f	Respiratory frequency

STPD Standard temperature, pressure, dry (0° C, 760 torr)
BTPS Body temperature, pressure, saturated with water
ATPS Ambient temperature, pressure, saturated with water

All the gas symbols are in subscript.
Dash above a symbol designates a mean value.

Examples

$F_{E_{N_2}}$	Fractional concentration of nitrogen in dry expired gas
$P_{C_{O_2}}$	Mean partial pressure of oxygen in capillary blood
$P_{A_{O_2}}—P_{a_{O_2}}$	Difference of the partial pressures of oxygen between alveolar gas and arterial blood
P_B	Barometric pressure
C_{vCO_2}	Concentration of carbon dioxide in venous blood
$S\bar{v}_{O_2}$	Saturation of oxygen in mixed venous blood
V_I	Inspired volume
V_T	Tidal volume
D_{LCO}	Pulmonary diffusing capacity for CO, *e.g.* 17 ml STPD · min^{-1} · $torr^{-1}$

CONTENTS

Y. L. Lai, E. D. Martin, B. A. Attebery and E. B. Brown, Jr.: Mechanisms of extracellular adjustments in hypercapnia 107
Y. L. Lai, B. A. Attebery and F. B. Brown, Jr.: Intracellular adjustments of skeletal muscle, heart, and brain to prolonged hypercapnia 115
Y. L. Lai, B. A. Attebery and E. B. Brown, Jr.: Mechanisms of cardiac muscle adjustment to hypercapnia 123
A. Toulmond: Tide-related changes of blood respiratory variables in the lugworm *Arenicola marina* (L.) 130
Patricia M. Tweeddale: Blood oxygen affinities of the adult and foetal large white pig 145
R. Baumann, F. Teischel, R. Zoch and H. Bartels: Changes in red cell 2,3-diphosphoglycerate concentration as cause of the postnatal decrease of pig blood oxygen affinity 153
Danuta T. Zborowska-Sluis and G. A. Klassen: Carbon dioxide mediated glycolysis II 162
P. J. Butler and E. W. Taylor: The effect of hypercapnic hypoxia, accompanied by different levels of lung ventilation, on heart rate in the duck . . 176
D. Kerem and R. Elsner: Cerebral tolerance to asphyxial hypoxia in the harbor seal 188
P. McN. Hill: Hyperventilation, breath holding and alveolar oxygen tension at the breaking point 201
J. Leigh, W. L. Walker and K. T. Fowler: Effect of hypnotic suggestions on the respiratory response to transient CO_2 breathing in man 210
R. Markello, A. Olszowka, P. Winter and L. Farhi: An up-dated method for determining $\dot{V}A/\dot{Q}$ inequalities and direct shunt using O_2, CO_2 and N_2 221
H. Slama, W. Schoedel and E. Hansen: Lung surfactant: film kinetics at the surface of an air bubble during prolonged oscillation of its volume . . . 233

SOME FORTHCOMING PAPERS

H. L. Barlett, J. Kollias, J. L. Hodgson and E. R. Buskirk: A possible explanation for exercise DL_{CO} based on estimations of SAL and τ_h
J. S. Brody and W. J. Buhain: Hormonal influence on post-pneumonectomy lung growth in the rat
J. H. Cameron and T. A. Mecklenburg: Aerial gas exchange in the coconut crab, *Birgus latro* with some notes on *Gecarcoidea lalandii*
D. M. Gomez and P. R. B. Caldwell: A formulation for the partition of free vs. hemoglobin-bound 2,3-Diphosphoglycerate
G. K. Snyder: Erythrocyte evolution: the significance of the Fåhraeus-Lindqvist phenomenon